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Methods of Diagnosing in Liver Diseases for Dog and Cats

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Abstract
Diagnosis of liver diseases can be difficult because the symptoms of the organ may be ambiguous or may easily interfere with the symptoms of other liver diseases. In some cases, the animal may have no symptoms, but the liver may already be severely damaged. Liver diseases have multiple causes including infectious, genetic, autoimmune, and some metabolic. This makes diagnosis of the disease difficult and needs to be done to correctly diagnose the underlying cause of the disease. Diagnosis of liver diseases is based on initial formation and physical examination. Findings of liver diseases; weakness, fatigue, swelling of the abdomen, pain and nausea. In addition to clinical findings to diagnose liver diseases, existing tests called liver tests such as blood tests, hepatic enzymes, protein metabolism, lipid metabolism, carbohydrate metabolism and bilirubin urine analysis are used as additional diagnostic tests and include abdominal radiography, abdominal ultrasonography, nuclear scintigraphy, Such as computerized tomography, magnetic resonance imaging, and kallistatin levels are commonly used tests in liver diseases.
In this review, detailed information about abdominal radiography, abdominal ultrasonography, nuclear scintigraphy, computed tomography, magnetic resonance imaging kallistatin and vitamin D levels will be given in addition to the routine tests used to diagnose liver diseases.
Keywords: Diagnosis, Disease, liver, Dog, Cat

INTRODUCTION

The liver is second largest organ in the body this organ has more biochemical functions than any other organ of the body which is mainly comprised of hepatocytes, sinusoidal cells, and biliary epithelium [1]. Hepatocytes make up approximately 60% of the hepatic parenchyma with sinusoidal endothelial cells, hepatic stellate cells (called Ito cells), liver-associated Kupffer cells and lymphocytes [2]. The approximation of the gutter-like hemicanals on adjacent surfaces of neighboring hepatocytes form the intercellular space called the *canaliculus*, which is the beginning of the hepatobiliary system. The hepatic artery and the portal vein are the two blood supplies of the liver which comprise nearly 20% and 80%, respectively, of the total blood circulation flow, which mixes as it enters the sinusoids. liver is performs an estimated 1500 essential biochemical functions. It functions in hundreds of diverse metabolic activities including synthesis of plasma proteins catabolism and storage of carbohydrates synthesis, degradation of lipids detoxification and excretion of many toxic agents the formation and elimination of bile [2].

Multiple tests have been developed to assess liver function because of this cellular, biliary, and vascular complexity [1]. These tests and examinations aim to evaluate hepatocyte function and membrane integrity, the portal circulation, hepatobiliary function, and the enterohepatic circulation. Due to the intimate anatomic and intertwining functional relationships of hepatic components, there is frequent overlap of findings with varied pathology of the liver, the portal circulation and extrahepatic diseases [2].

Hepatic function (Figure1) shows the complex cell constituency comprising the liver and the microanatomic

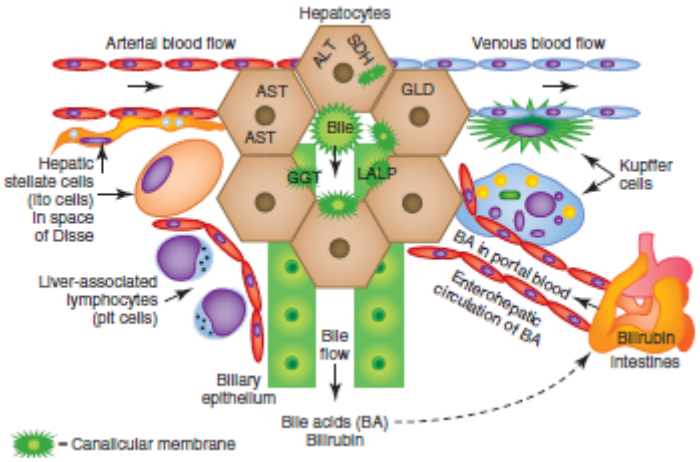

Figure1. Hepatic functions

Table1. Cells of the liver and their functions

Cell Type	Other Name	Function	Cell Markers
Hepatocytes	Liver cells	Intermediary metabolism	Albumin, cytokeratin 8 and 18
Cholangiocytes	Biliary epithelial cells	Line the bile ducts, secretion	Cytokeratin 7 and 19
Kupffer cells	Browicz-Kupffer cells, stellate, macrophages	Phagocytosis of pathogens and particles	ED-1 and ED-2
Stellate cells	Ito cells, vitamin A–storing cells, lipocytes	Storage of vitamin A; production of myofibroblasts in injury	GFAP, desmin; α-smooth muscle actin
Natural killer (NK) cells	Pit cells, large granular lymphocytes, γδ T cells	Immune surveillance infection, cancer	CD3
Vascular endothelial cells	Endothelial cells	Line blood vessels	CD34 and CD31
Lymphatic endothelial cells	Endothelial cells	Line lymphatic vessels	Podoplanin
Smooth muscle cells	Myocytes	Regulation of Microcirculation	Myocardin, α-smooth muscle actin
Stem cells	Progenitor cells, oval cells	Bi-potential progenitor cell for hepatocytes and biliary epithelial cells	α-Fetoprotein
Portal tract fibroblast	Fibroblasts	Integrity of portal triads, supporting function	Vimentin

relationships of these cells, the cellular location of liver enzymes, and the enterohepatic circulation of bile acids [3]. When evaluating a patient animal with suspected hepatobiliary disease signs, clinical history beside the physical examination can help to formulate a list of reasonable differential diagnoses [2, 3].

Diagnosis of Liver Disease

Diagnostic Strategy

Diagnostic evaluation of the hepatobiliary system has several aims.

To determine if hepatobiliary disease is present

To assess liver function

To definitively diagnose hepatobiliary disease

To monitor response to treatment [4].

History

A properly taken history is pivotal to defining the most clinically relevant problems that need to be resolved [3, 4, 5]. A structured interview process and understanding the basics of communication are important success factors to retrieve this crucial information [5]. Fortunately, the knowledge about communication in the medical profession and the focus on the veterinary curriculum, has increased considerably during the last few years some basic principles should be kept in mind to understand the symptoms in dogs and cats with diseases affecting the hepatic parenchyma, portal vasculature, and the biliary system [4, 5]. First, for most of its functions, the liver has a tremendous (approximately 80 %) reserve capacity and a remarkable potential to regenerate. Symptoms occur only when progressive disease exhausts hepatic reserves. Diseases often remain subclinical for lengthy periods of time; symptoms may be relatively mild and nonspecific because the liver reserve prevents overt abnormalities. Symptoms such as lethargy, vomiting, or mild polyuria and polydipsia (PU/PD) may alert the clinician that a liver disorder could be developing [3, 5].

Clinical Examination

Removal of exogenous and endogenous toxins synthesis of vital substances such as albumin and blood clotting factors protein fat and carbohydrate metabolism vitamin storage and activation glycogen and triglyceride and mineral storage; activation, conversion, secretion, deactivation, and excretion of various hormones; bile salt synthesis; conjugation and excretion of bilirubin in bile [6, 7].

LABORATORY FINDINGS

Hepatic Enzymology

Three principal factors contribute to normal serum hepatobiliary enzyme activity. The first determinant is the normal concentration of that enzyme in tissues. Enzymes must be present in a high enough concentration for some spillover into the circulation to occur. The second determinant is the serum enzyme's half-life. An enzyme must have a serum half-life of sufficient duration to permit accumulation. The final determinant is intracellular localization, since enzymes must have access to the vascular compartment to be measured in serum [6]. In general, cytosolic enzymes gain access to the serum easier than do enzymes that are within organelles or that are membrane-bound. Serum hepatobiliary enzyme activity increases because of leakage

Table 2. Clinical signs associated with liver disease

Depression	Weakness	CNS signs, Icterus
Anorexia	Vomiting	Change in spleen size
Diarrhea	Weight loss	Dark brown urine
Fever	Polydipsia	Polyuria
Abdominal pain	Ascites	Coma, Change in liver size
Dark or light color stools	Hemorrhage	Pruritus

from damaged hepatobiliary cells, elution from damaged membranes, or increased synthesis. Serum hepatobiliary enzyme activity measurements are useful screening tests for liver damage. They have a high sensitivity (a measure of a test's ability to detect animals with hepatobiliary disease), so few patients with liver disease are missed (the false negative result rate is low). However, they have a lower specificity (a measure of a test's ability to exclude individuals without hepatobiliary disease), so some animals without liver disease will have elevations (false positive results). Thus, once an elevation in serum hepatobiliary enzyme activity is noted, confirmation of hepatobiliary disease requires performing tests with higher specificity [6, 8]. Abnormal liver enzymes are so important and should be investigated in a systematic manner. The survey of abnormal biochemical tests of liver in the asymptomatic and the symptomatic patient is a common finding on a normal blood screen [8]. ALT serum rasing are specific remarked in dogs. ALT activity can raise with muscle injury, but simultaneous evaluation of CK activity can help to unapt the muscle source [6, 9] raising in serum ALT activity have the highest sensitivity (more than %80) for hepatic disorders but have less sensitivity (under 60 %) in cases of hepatic congestion, neoplasia, and portosystemic vascular anomalies [6, 9]. ALP serum raising activity is one of the most common problems detected in ill dogs. ALP activity measurement has a high sensitivity (80%) for hepatobiliary disease, but its specificity is low (50%). If elevated ALP activity is noted with a concurrent increase in serum GGT activity, specificity for liver disease increases up to 90 % [10]. In the liver abnormalities biochemical identification should suggest definit diagnostic possibilities that should guide a protocol for further examination [9, 11]. Liver biochemical abnormalities are often nonspecific; the measured enzymes can be the same enzyme from a different tissue source or isoenzymes from another tissue. In order to understanding of the liver biochemical tests is essential when evaluating the sick animal in question. Biochemical test due to liver abnormalities are divided into three branches that are **a**: cholestasis, **b**: hepatocellular injury **c**: tests of impaired metabolic function or synthetic capacity [8, 9, 10]. Hepatic enzymes can be divided into markers of hepatocellular damage and markers of cholestasis in animal with hepatocellular injury increases in either ALT or aspartate aminotransferase (AST) increase and their activity demonstrate leakage of the enzymes and hepatocellular membrane damage. AST serum rasing in the absence of increased ALT activity, show an extrahepatic problems, like muscle injury [9]. Measuring AST activity is sensitive but less specific for detecting hepatic disease than is measuring ALT serum activity there is high amount of ALT in hepatocyte cytoplasm of canine and it contain low amounts of AST [6]. Altered permeability of the hepatocellular membrane caused by damage or a metabolic disorder due to release this soluble enzyme. Conceptually, both of ALT and AST levels must be considered as hepatocellular "leakage" enzymes. Subsequent to an acute, diffuse injury, the magnitude of increase crudely reflects the number of affected hepatocytes. It is, however, neither specific for the cause of liver disease nor predictive of the outcome. The serum half-life of ALT is generally believed to be shorter in the cat than in the dog plasma half-life of ALT activity is 60 hours in dogs; however, ALT concentrations may take days to weeks to decrease following an acute insult [6, 9, 10]. As ALT is metabolized in the liver its serum half-life may be longer in patients with liver disease in the dog when persistent increases of ALT are characteristic of chronic hepatitis in the dog. ALT increases should be investigated when they are greater than twice the normal or persistently abnormal. ALP is considered to be a sensitive marker for cholestasis with a sensitivity of 85%. The short half-life of ALP in cats means that increases in ALP during cholestasis are not as high as in the dog. Consequently, ALP is a less-sensitive marker of cholestasis in the cat than in the dog, with a reported sensitivity of only 48% [9]. Changes in serum gamma-glutamyltransferase (GGT) activity generally parallel those in serum ALP activity, in that activity is often increased in patients with cholestasis. Cats with hepatic lipidosis may be an exception to this as they often have a normal serum GGT activity but an increased serum ALP activity. Skeletal muscle and liver, contains large amount of AST activity and nearly 80 % of AST is located predominately in hepatocyte mitochondria [11]. GGT serum measuring have a lower sensitivity but higher specificity (87%) for detecting liver disease than ALP serum activity [10]. Severe raising in GGT activity have importance in diseases of the biliary epithelium such as bile duct obstruction and cholecystitis [6]. Intermediate elevations are indicated primary hepatic neoplasia (hepatocellular and biliary carcinoma) and corticosteroid induction [6, 10, 12]. low elevations are indicated hepatic necrosis and anticonvulsant administration [6, 13]. In a case of injury in skeletal muscle serum AST ant ALT increasing but its notable that increase amount of ALT to a much lesser extent. ALT activity can be further defined as muscular in origin by the measurement of serum creatine kinase (CK) which is specific muscle enzyme. Clinical experience in veterinary medicine demonstrate that there is value in the interpretation of the serum activities of ALT and AST for liver disease. The half-life for ALT is nearly 2.5 days. After an acute damage and injury bring about a moderate to marked increase in the serum AST and ALT concentrations in serum, the amount of AST serum will return to normal more rapidly (hours to days) than the ALT serum (days), due to their difference in plasma half-life and cellular location [11, 12]. Tests like drug induction ALP and cholestasis and GGT show minimom activity in healthy hepatic tissue but it can be increased in the serum due to increased enzyme production stimulated by either drug induction or impaired bile flow [11].

Table 3. Approximate half-life of serum hepatic enzyme activities (hours) for the dogs and cats [3].

Enzyme	Dog	Cat
Alanine aminotranserase (ALT)	60	3.5
Glutamine dehydrogenase (GLD)	18	Not determined
Aspartate aminotransferase (AST)	12	1.5
Alkaline phosphatase (ALP)	66	6

As mentioned before ALT and AST are increasing in a case of hepatocellular leakage where as ALP and GGT are increasing in a case of cholestasis. As ALT is metabolized in the liver its serum half-life may be longer in patients with liver disease in the dog. ALP is considered to be a sensitive marker for cholestasis with a sensitivity of 85%. The short half-life of ALP in cats means that increases in ALP during

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Plasma Proteins

The measurement of plasma albumin and prothrombin time may be used to assess function. The hepatic synthetic and secretory capacities are large; only severe and usually prolonged liver disease, for example cirrhosis, demonstrably impairs albumin and prothrombin synthesis [17, 18].

Markers of Protein Metabolism

The liver plays a central role in protein metabolism. It is responsible for synthesis of plasma proteins, deamination of amino acids, conversion of ammonia to urea, amino acid

Table 4. When Serum transferase levels may not reflect clinical hepatobiliary disease

Endocrinopathies	Diabetes mellitus Hyperadrenocorticism Hyperthyroidism(cats)	Hyperthyroidism(cats):↑ALP, ↑AST Hyperthyroidism(dogs):↑ALP Diabetes mellitus:↑ALP Hyperadrenocorticism(dogs) ↑↑↑ALP, ↑AST, ↑ALT, ↑GGT
Gastrointestinal Disease	Pancreatitis Inflammatory bowel disease(IBD)	Genetics, nutrition, infectious agents, and abnormalities of the immune system may all play a role. Inflammatory bowel disease may not be an actual disease onto itself, but a characteristic response of the body to certain conditions caused by a variety of factors in dogs and cats. N, ALP, ↑AST, ↑ALT, ↑GGT
Neoplasia	Metastatic disease	Adenocarcinomas, pancreatic sarcoma, intestinal sarcoma, adernocortical sarcoma, mammary sarcoma, Hemangiosarcoma, Leiomyosarcoma, Hepatic metastasis. (↑ALT, ↑AST, ↑ALP) Unique enzyme induction. (↑↑ALP, ↑GGT)
Drug induction	Phenobarbital (dogs) Corticosteroids (dogs)	Corticosteroids (dogs). ↑↑↑ALP, ↑↑GGT, ↑ALT, ↑AST Anticonvulsants. Phenobarbital, primidone. ↑ALT, ↑ALP, ↑ AST, ↑GGT
Hypoxia/hypotension	Congestive heart failure Hypotensive crisis Severe hemolytic anemia Status epilepticus	Congestive heart failure Septic shock Hyperadernocorticism Circulatory shock Severe acute blood loss Status epilepticus Hypotensive crisis, Surgery ↑↑ALT, ↑ ALP, ↑GGT, ↑ AST
Muscle injury		Acute muscle necrosis or truma Malignant hyperthermia Myopathies ↑ALT, ↑ ↑AST
Bone disorders		Young animal :↑ALP Osteosarcoma:↑ALP Osteomyelitis:↑ALP
Miscellaneous	Systemic infections	Pregnancy (cats):↑ALP Colostrums fed neonates (dogs):↑GGT

↑: raised, **N:** normal**, ↓:** reduced

synthesis, and inter conversion of amino acids [19].

Albumin

Albumin is an important plasma protein that is produced exclusively by the liver. It is possible to determine the cause of severe hypoalbuminemia from a combination of clinical findings, measurement of the serum globulin concentration, urinalysis (including protein creatinine ratio), tests of gastrointestinal protein loss, and tests of liver function but it is a less specific indicator of impaired synthetic capacity than a prolonged prothrombin time. A plasma albumin concentration below the lower reference limit may simply hepatic disease chronicity. However, there are many other causes of a low plasma albumin concentration that are not due to hepatic disease. The rate of albumin synthesis must equal the rate of albumin loss to maintain serum albumin concentrations. Mild decreases in serum albumin concentration can occur from a variety of conditions. However, the differential diagnoses for severe hypoalbuminemia (<2 g/dL) are limited to hepatic insufficiency, severe exudative skin disease, protein-losing enteropathy, and protein losing nephropathy [19].

As albumin contributes significantly to colloid oncotic pressure severe hypoalbuminemia can lead to ascites, pleural effusion, and/or subcutaneous edema. Albumin In normal case The rate of albumin synthesis must equal the rate of albumin loss to maintain serum albumin concentrations The rate of albumin synthesis must equal the rate of albumin loss to maintain serum albumin concentrations. Mild decreases in serum albumin concentration can occur from a variety of conditions [20]. The liver has a large reserve capacity for the synthesis of albumin and albumin has a serum halflife of approximately 7 days in dogs [21]. Consequently, hypoalbuminemia is a relatively insensitive marker for hepatic insufficiency and is only likely to be seen in patients with advanced chronic liver disease or portosystemic shunts (PSSs) [20, 21].

Prothrombin Time

The majority of coagulation factors are synthesized in the liver. The liver have important role in the producing of anticoagulant factors, products of fibrinolysis and the removal of activated clotting factors [22, 23,24]. Prothrombin time (PT) and activated partial thromboplastin time (aPTT) must be majured before the procedures that compromise vessel integrity, it is important to perform liver biopsy in a case of hepatic pathology is suspected. In acute hepatic necrosis aPTT and PT are prolonged [22] whereas only aPTT remains prolong in dogs with congenital portosystemic vascula problems [23, 24]. Coagulation factors II, VII, IX, and X have a relationship with vitamin K for activity. Vitamin K deficiency and the verisimilitude of bleeding are associated with prolonged anorexia and bile duct obstruction fore example hepatic lipidosis in cats. Measurement of protein induced by vitamin K absence or antagonists (PIVKAs) become manifest to be more sensitive for the detection of vitamin K deficiency than measuring the PT and aPTT [25]. The PT may be prolonged by cholestasis, fat-soluble vitamin K can not be absorbed normally if fat absorption is impaired due to bile salt leakage of intestinal. The deficiency can be solved by parenteral administration of the vitamin K. When vitamin K deficiency is the cause of the abnormal coagulation tests, these return to reference values within 24 to 36 hours following the parenteral administration of vitamin K, but remain abnormal if the activity of other coagulation factors is reduced because of liver pathology [25]. A prolonged prothrombin time may also result from severe impairment of synthetic ability if the liver cell mass is decreased in this cases it is not solved by parenteral administration of vitamin K [24, 25].

Table 5. Typical biochemical features of certain hepatic disorders

Liver Diseases	Plasma albumin	Bilirubin	ALT	ALP	GGT
Acute alcoholic hepatitis	-	↑	↑	↑	↑↑
Acute viral hepatitis	-	↑↑	↑↑	↑	↑↑
Chronic viral hepatitis	↓or N	↑or N	↑	↑or-	↑
Cirrhosis	↓	↑or N	↑	↑	↑
Primary biliary cirrhosis	↓or N	↑↑	↑	↑↑	↑↑
Tumour secondaries	N	↑or N	↑	↑↑	↑↑

↑: raised, **N**: normal, ↓: reduced

ALT: Alanine aminotransferase, **ALP**: Alkaline phosphatase, **GGT**: ᵞ-glutamyl transferase

Globulin

Globulins are produced in the liver, but not exclusively so. The liver produces α-globulins and β-globulins, whereas lymphoid cells produce immunoglobulins (γ-globulins). Hepatic insufficiency rarely leads to a decrease in serum globulin concentration [20].

Acute Phase Protein

The acute phase refers to nonspecific inflammatory reaction of the animal that occurs shortly after any tissue injury [25, 26]. Plasma proteins called acute phase proteins (APPs) are plasma proteins which is changed in concentration such as decrease in albumin or transferrin, and increase in concentration such as serum amyloid A, haptoglobin, C-reactive protein, ceruloplasmin and alpha-1-acid glycoprotein. The acute phase response and clinical application of monitoring APPs in dogs and cats are diagnosed by a numerous of different systemic effects, such as fever, increased blood cortisol and decreased thyroxine concentrations, metabolic changes, leukocytosis [26].

Protein Catabolism

Ammonia is produced in the intestinal and carried by the portal blood flow to the liver metabolize. One by-product is urea nitrogen, which enters the blood flow and is excreted by the renal system. Hyperammonemia is related to portosystemic shunting because of portosystemic vascular problems that can be acquired secondary to

cirrhosis or congenital. Hyperammonemia also facilitates the development of ammonium biurate crystalluria, which is commonly associated with congenital portosystemic vascular anomalies. The finding of hyperammonemia supports the clinical suspicion of hepatic encephalopathy. the the measurement of the fasting plasma ammonia concentration was more notable and specific diagnose in dogs with portosystemic shunts than fasting plasma bile acid concentration [27]. Ammonia can be measured before and after the administration of ammonium chloride per os or per rectum. Blood is collected into ammonia-free heparinized tubes and blood ammonia measured [28] point is considered abnormal if ammonia increase more than three times. The sensitivity of plasma ammonia measurement for the detection of portosystemic shunt (PSS) is reported to be between 81% and 100% in dogs but 83% in cats. The extraction of ammonia from the portal circulation is highly efficient. Endogenous ammonia is produced from the breakdown of nitrogenous substances in the body, especially glutamine. In the liver the ammonium is converted to urea by the enzymes of the urea cycle, or is used during the conversion of glutamate to glutamine [29]. Urea is produced from ammonia in the liver, released into the systemic circulation, and subsequently excreted by the kidneys. Serum urea nitrogen concentration may be close to or below the lower limit of the reference interval in patients with hepatic insufficiency [30]**.** In dogs and cats of increasing the level of amonemia caused by an enzyme deficiency in the urea enzyme cycle rarley have been reprted [32]. Clinical signs of liver encephalopathy in dogs and cats are associated with an ammonia disturbance tolerance test but a normal serumconcentration of whole bile acids [30, 31].

Carbohydrate Metabolism

The liver plays a central role in carbohydrate metabolism and is responsible glycogen storage, conversion of galactose and fructose into glucose, gluconeogenesis, and the synthesis of many compounds from carbohydrates [32]. Animals with Liver diseases are susceptible to have accompanying diabetes mellitus. Blood pyruvate in these animals beside total bilirubin and globulin levels were raised, but serum albumin level decreased significantly whilst concentrations of blood glucose, total lipids, total proteins, serum cholesterol and urea remained normal carbohydrate metabolism is disturbed in liver insufficiency which may lead to diabetes mellitus [33].

Plasma/Serum Lipid

The liver plays a central role in lipid metabolism and is responsible for oxidation of fatty acids, synthesis of cholesterol, synthesis of lipoproteins, and synthesis of fatty acids from proteins and carbohydrates [32]. Hepatocytes produce primary bile acids from cholesterol which are secreted by the canalicular membrane into canaliculi for transport to the intestinal tract by the biliary system after that chenodeoxycholic acid and cholic acid conjugate them to glycine or taurine. Conjugated bile acids are approximately totally absorbed (~95%) by the ileum into the portal blood flow and carried back to the liver for efficient first-pass absorb (70% to 80%) by hepatocytes that primarily located in periportal area [34, 35]. Conjugated bile acids are again excreted into the biliary system for another enterohepatic journey during which they contribute the osmotic force that obliges bile circulation and provide surface-active detergent attributes that makes intestinal easily absorption of lipids. This recycling of bile acids is due to as an enterohepatic circulation system. Tinny quantities of early bile acids that are not reabsorbed in the ileum are dehydroxylated by anaerobic bacteria in the colon to form secondary bile acids. Some of these are absorbed into the portal venous flow and again recycled. Conjugated early and secondary bile acids contain the large amount of the bile acid in the portal circulation. Bile acids grasp by hepatocytes from the sinusoidal blood covers the sodium taurocholate cotransporting protein (NTCP) and the organic anion transporting protein (OATP), and bile acid carried into the bile at the canalicular domain is driven by the bile salt export pump (BSEP) [31, 36]. Bile acid concentrations are usually measured after prevention food 12 hours and again approximately 2 hours after a small meal of canned pet food in order to stimulate contraction of the gallbladder and stimulate the liver function [36]. To elude lipemia, 2 to 3 tea spoons are given to animals weighing less than 12 pounds and up to 3 tables poons are given to heavy animals. After meal contraction of gallbladder is rather weak or may occur during the fasting period, consequence of fasted value that exceeds the postprandial value. Both values are usually normal and within the reference interval. Total bile acid concentrations increase in the circulation when pathology varies the enterohepatic circulation. Serum bile acid in a case of fasting or postprandial concentrations approximately higher than and 25 µmol per Liter for the dogs and 15 µmol per Liter for the cats are supportive of hepatic pathology or portosystemic vascular disorders [14, 37]. Bile acids can easily measure in the urine of dogs and cats [38, 39]. Hepatic biopsy and microscopic survey is essential to diagnose the liver pathology and designate its severity. In a case of occurring unusual ammonia endurance test a congenital portosystemic vascular anomaly is indicated. Small intestinal bacterial overgrowth (SIBO) can increase the deconjugation of bile acids, which are less efficiently take out from the portal flow resulting in an increase in the circulating total bile acid. An increase of a specific unconjugated bile acid, unconjugated cholic acid has been associated with SIBO in dogs [40, 41]. Hypoglycemia is one of the diagnostic parameters caused by liver disease which is often associated with congenital portosystemic vascular disorders, in small breeds and in patients with acute hepatocellular necrosis [38, 42]. Hypoglycemia is also associated with glycogen storage insufficiency and early hepatic neoplasia [43, 44, 45, 46]. Liver-related hypocholesterolemia is often associated with congenital portosystemic vascular problems [42]. Serum cholesterol concentrations may be increased, normal, or decreased in patients with liver disease. Increased or decreased fasting serum cholesterol concentrations are not sensitive or specific for hepatobiliary disease in dogs or cats. There is some evidence that hypertriglyceridemia is associated with gallbladder mucocele formation [47]. Hypertriglyceridemia is associated with increased serum hepatic enzyme activities in Miniature Schnauzers [42]. Serum triglyceride concentration may be increased or normal in patients with liver disease. However, an increased fasting serum triglyceride concentration is not a sensitive or specific marker for hepatobiliary disease in dogs or cats because they are also observed in patients with endocrinopathies, obesity, pancreatitis, and primary hyperlipidemias [42, 47].

Bilirubin

Bilirubin is a yellow pigment produced by the breakdown of heme-containing compounds (aged erythrocytes by macrophages) and caried to the liver [46]. Measurement of serum bilirubin concentration can be used to assess liver function. This substance is called unconjugate bilirubin [48, 49]. Liver cells totally remove and conjugate it with glucuronic acid by the uridine diphosphate glucuronosyltransferase 1 (UGT1A1) which is an enzyme in the uridine diphosphate glucuronosyltransferase (UGT) groups [48, 49, 50]. Some drugs impair the function of UGT1A1, due to an increase in unconjugated bilirubin in the blood flow without anemia or hepatic pathology [50, 51]. Bilirubin is derived from the metabolism of aged erythrocytes by macrophages and transported to the liver [52]. This metabolite is refer to as unconjugated (indirect reaction) bilirubin. Hepatocytes officiently remove it from the sinusoidal blood and conjugagte it with glucuronic acid: a process that is catalyzed by UGT1A1. Conjugated bilirubin is excreted into the bile and carried to the intestinal where it is converted to urobilinogens and stercobilin by bacteria, the latter imparts the brown color to feces. Measurement of urinary urobilinogen is an old test that was help to diagnose of bile duct patency at the moment clinically is considered useless [50, 51]. A variety of bacterial infections can release harmful toxins that can effected canalicular membrane function, due to retention of conjugated bilirubin in the blood flow but there is no physical biliary obstruction and it is thought a functional impairment of bile flow. Often there is only minimal concomitant change in liver enzyme activities [52, 53] increase in bilirubin resolves with successful management of the extrahepatic infection. Blood urea nitrogen (BUN) is a by-product of ammonia metabolism. BUN is below the reference interval or inappropriately low compared with creatinine in most dogs and cats with congenital portosystemic vascular anomalies as a result of shunting of rich-ammonia portal blood directly into the blood flow [54]. Hyperbilirubinemia can be the result of hepatobiliary or extrahepatic disease. Serum bile acids (SBAs) measurement is a useful test of liver function in dogs and cats. SBAs are either measured as a fasting sample (after withholding food for 12 hours) or by collecting paired fasting and 2-hour postprandial samples. When exposed to air the urobilinogen remaining in the intestines is altered and oxidized into the brown pigment sterocobilin [4]. Prehepatic hyperbilirubinemia is caused by increased production of bilirubin as a result of hemolysis. The liver has a large reserve capacity for bilirubin excretion so, for hemolysis to cause hyperbilirubinemia, hepatic bilirubin clearance must be decreased [55]. This occurs if the hemolytic anemia results in hepatocyte dysfunction because of hypoxia. Increased SBA concentrations (fasting or postprandial) suggest hepatic dysfunction, PSS, or cholestasis, but they are not specific for any particular liver disease. Hyperbilirubinemia leads to icterus. The kinetics of bilirubin process of dog and cat is differing than human [54]. Conjugated bilirubin can appear in the urine (bilirubinuria) and a tiny amount of conjugated bilirubin can be detected in healthy dogs. Measurement serum bilirubin isn't helpful in determining the cause of hyperbilirubinemia. A rapid destruction of erythrocyte because of severe hemolytic disease due to increase in bilirubin is associated with anemia. Liver enzyme activities may also be abnormal in these patients because of the anemia. Separation of hyperbilirubinemia because of hepatic icterus versus obstruction of the bile duct is challenging. Ultrasound and often liver biopsy are used to diagnose bile duct patency. Variable amounts of a second type of conjugated bilirubin can form with prolonged cholestasis. Conjugated bilirubin has bound with albumin. This type of bilirubin is called biliprotein or δ-bilirubin. Once, the cholestatic process is resolved, δ-bilirubin is removed from the circulation in 7 to 8 days. Following successful management If the majority of conjugated hyperbilirubinemia is in the form of biliprotein, icterus will be protracted (days to weeks) [55].

Urinalysis

Urine specific gravity can be decreased in patients with hepatic insufficiency or portosystemic shunts (PSSs). This can be caused by an inability to fully concentrate urine, resulting in polyuri (PU), or from primary PD in dogs Bilirubinuria (<2+ on a dipstick) can be a normal finding in dogs [56]. Cats have a higher renal threshold for bilirubin than dogs, bilirubinuria should always be considered abnormal in cats. Bilirubinuria in cats and excessive bilirubinuria in dogs implies hemolytic or hepatobiliary disease. Uric acid is a product of purine catabolism and is converted to allantoic acid by hepatic urate oxidase so in cases with severe hepatic insufficiency or PSS, the serum uric acid concentration may be higher than the renal threshold [56]. Urate urolithiasis seems to be more common in patients with PSS than those with other types of hepatic dysfunction. Between 40% and 70% of dogs with PSS were found to have urate crystalluria. However, it should be noted that urate crystalluria is not specific for hepatobiliary disease [13].

Hematology

Patients with hepatobiliary disease can be anemic as a result of blood loss, dysmorphias and anemia. Red blood cell morphologic changes are sometimes observed in dogs with hepatobiliary disease Poikilocytosis, may be seen in patients with chronic hepatic disease. Patients with PSS can have microcytic red blood cells. This is more common in dogs than in cats Microangiopathy can occur as a result of hepatic neoplasia or DIC, and may lead to the formation of schistocytes [57,58]. The thrombocyte series is occasionally affected by hepatobiliary disease, but changes are both inconsistent and nonspecific. Mild to moderate thrombocytopenia may occur in patients with severe liver disease [57]. This may be the result of a decreased production of thrombopoietin by the liver. Disseminated intravascular coagulopathy (DIC) associated with liver disease also may lead to thrombocytopenia. Additionally, infectious diseases affecting the liver, such as leptospirosis may result in thrombocytopenia [58].

Histopathologic Analysis

Histopathologic analysis of liver biopsies or identification of a shunting blood vessel is often required to definitively diagnose hepatic disease [45, 59]. However, the pattern of laboratory test abnormalities, particularly when interpreted in conjunction with the patient's clinical presentation, and the results of diagnostic imaging, can increase or decrease a clinician's index of suspicion for specific liver diseases [45, 60, 61, 62, 63].

Diagnostic Imaging in Liver Diseases

Diagnostic imaging may help to determine; whether or not hepatobiliary disease is present, Identify the cause of

Table 6. Typical patterns of clinicopathologic changes associated with liver disease in the dogs and cats [45].

Laboratory Test	Acute Hepatitis /Hepatic Necrosis	Chronic Hepatitis	Cirrhosis	CPSS	Biliary Tract Obstruction	Nonobstructive Biliary Tract Disease	Hepatic Neoplasia
ALT	↑↑-↑↑↑	↑-↑↑↑	N-↑↑	N-↑	N-↑↑	N-↑↑	N-↑↑
ALP	↑-↑↑	↑-↑↑	N-↑↑	N-↑	↑↑↑	↑-↑↑↑	N-↑↑
Total bilirubin	N-↑↑↑	N-↑↑	N-↑↑↑	N	↑↑-↑↑↑	N	N-↑
Preprandial SBA	N-↑↑	N-↑↑	↑-↑↑↑	N-↑↑	↑↑-↑↑↑	N	N-↑
Postprandial SBA	N-↑↑	N-↑↑	↑-↑↑↑	↑↑-↑↑↑	↑↑-↑↑↑	N	N-↑
Ammonia	N-↑↑	N-↑↑	N-↑↑	↑-↑↑↑	N	N	N-↑

↑: Mild increase. ↑↑: moderate increase, ↑↑↑: severe increase, ALP: serum alkaline phosphatase activity; ALT: serum alanine aminotransferase activity; CPSS: congenital portosystemic shunt; N: within the reference interval, SBA: serum bile acid concentration.

a secondary hepatopathy, aid in the diagnosis of specific hepatobiliary diseases, Provide prognostic information, and with the exception of diagnosis of a PSS. Radiography and abdominal ultrasound are the most frequently used imaging modalities for assessment of the hepatobiliary system in dogs and cats, but alternative imaging techniques are now being used more frequently [64, 65].

Abdominal Radiographs

Abdominal radiographs allow assessment of hepatic size, shape, opacity, and location in most patients [60, 61, 65]. Radiography may also allow identification of extrahepatic abnormalities that affect the liver. However, radiographs provide limited information about the hepatic parenchyma [66]. It is important to note that patients with hepatobiliary disease often have normal abdominal radiographs. Radiography allows subjective assessment of liver size. Hepatic size, shape, opacity, and location in most patients may also allow identification of extrahepatic abnormalities that affect the liver Radiolucent areas within the liver indicate accumulation of gas within the hepatic parenchyma, biliary tract, or portal vasculature Cranial displacement of the gastric axis may be observed on lateral abdominal radiographs when microhepatia is present [60, 61, 65, 66, 67].

Abdominal Ultrasound

Ultrasonography allows assessment of the hepatic parenchyma and biliary tract. Evidence of extrahepatic disease causing a secondary hepatopathy may also be detected hepatomegaly and microhepatia. In one study the overall accuracy of ultrasound for discrimination among different categories of diffuse liver disease was 36.5% for dogs and 54.6% for cats. Hepatic lipidosis in cats could be diagnosed slightly more accurately than other diffuse hepatic diseases [62, 63, 67]. Cytologic or histologic evaluation of a hepatic tissue sample is usually needed to make a definitive diagnosis [62, 63, 68].

Nuclear Scintigraphy

Nuclear scintigraphy involves administering a radioactive tracer substance (radiopharmaceutical) to the patient, which localizes to a specific organ or tissue. radioactive decay of this substance is detected by a gamma camera and used to form images [69]. Technetium-99m is the most commonly used radiopharmaceutical for assessing the portal circulation of small animal patients. By analyzing the radiation emitted from regions of interest drawn over the patient's liver and heart. Transsplenic portal scintigraphy is 100% sensitive and specific for the diagnosis of congenital PSS, and significantly more likely than per-rectal portal scintigraphy to detect shunt number and termination in dogs [47, 64, 69]. Nuclear scintigraphy has been used to quantify liver function and to assess biliary tract patency in dogs. In a retrospective study hepatobiliary scintigraphy was found to be 83% sensitive and 94% specific for the detection of extrahepatic biliary obstruction in dogs and cats [49, 64, 69].

Computed Tomography and Magnetic Resonance Imaging

Computed tomography (CT) and magnetic resonance imaging (MRI) have been used to detect hepatic parenchymal neoplasia [70]. Abdominal computed tomography image of a dog with a massive hepatocellular carcinoma [46,70]. There is a large irregularly shaped mass associated with the ventrolateral right side of the liver. Computed tomography (CT) and magnetic resonance imaging (MRI) have been used to detect hepatic parenchymal neoplasia in humans [71]. Compared to abdominal ultrasound these techniques have an improved accuracy for the diagnosis of hepatic neoplasia in humans. However, there is limited data in the veterinary literature evaluating their diagnostic performance. In one study, the diagnostic accuracy of CT for detecting hepatic masses was not found to be significantly different from that of abdominal ultrasound in dogs [70, 71]. In another study, MRI was found to have a sensitivity of 100% and a specificity of 86% for the differentiation between benign and malignant liver lesions in dogs (46). CT angiography is being used increasingly in dogs for the diagnosis of congenital PSS and other hepatic vascular diseases [46, 70, 71].

KALLISTATIN

Kallistatin is a protein that in animals is produced by the SERPINA4 gene which is serine proteinase inhibitor also called tissue kallikrein inhibitor. It binds tightly to tissue kallikrein but weakly to other serine proteinases such as chymotrypsin and elastase [66]. It is a new and reliable biomarker for the diagnosis of liver cirrhosis. Demonstrating a close correlation between the reduction in serum kallistatin levels and severity of early hepatic disease. Serum kallistatin levels could therefore provide an additional biomarker for the detection of LC and progressive loss of liver function along in response to the therapy. The data presented in this study showed that serum kallistatin levels in patients with LC were significantly lower than those in healthy controls, demonstrating a close correlation between the reduction

in serum kallistatin levels and severity of early hepatic disease. Serum kallistatin levels could therefore provide an additional biomarker for the detection of LC and progressive loss of liver function along in response to the therapy. The biology of LC is characterized by a constant stimulus for hepatocellular regeneration by a microenvironment associated with chronic inflammation and tissue fibrosis. Cirrhosis represents the final common pathological outcome for the majority of chronic liver diseases [70].

VITAMIN D

New İnsight of vitamin D in chronic liver diseases. Vitamin D is a fat-soluble sterol derivative that is predominantly synthesized in the liver and has multiple functions [71]. The accumulative data showed that the clinical manifestations and prognosis of chronic liver diseases are associated with serum vitamin D levels. The insufficiency or deficiency of vitamin D is common in various kinds of chronic liver diseases including viral hepatitis B and C. Serum 25-hydroxyvitamin D and vitamin D receptors are possibly interrelated with the incidence, treatment and prognosis of diseases [71, 72, 73]. Though the evidence of vitamin D supplementation in viral hepatitis and associated liver diseases is still limited, there is great potential to apply this adjuvant therapy to improve the treatments. Although the exact role and mechanisms of vitamin D have not been fully elucidated in chronic liver diseases, it is potentially beneficial in the treatment of chronic liver diseases. Further mechanistic studies are needed to validate its clinical application [44, 73, 74, 75].

In conclusion; a single liver test like laboratory test is of little value in screening and diagnosing for liver disease Once the liver disease has been categorized, following appropriate diagnostic tests beside a good history and physical examination are the most definitive and the most reliable ways to obtain the correct diagnosis of liver disease in dogs and cats.

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